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Original Article

Drug Resistant Tuberculosis: Pattern Seen among Patients Visiting Mayo Hospital, Lahore

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Abstract

Background: Multi drug resistant tuberculosis (MDR-TB) has become a challenge in management of TB. Especially with emergence of drug resistance (DR) over the last decade, it has created ambiguity among healthcare professionals treating this disease and anxiety among patients suffering from drug resistant TB. Thus, increase in drug resistance TB is alarming and reflecting various personal negligence level deficiencies. Continuous monitoring of drug resistance pattern is of great importance.
Objective: To see the shifting trends of drug susceptibility patterns among TB patients and to compare the frequencies of drug resistance among primary and acquired TB patients
Materials and Methods: This descriptive study was undertaken in Pakistan Health Research Council TB Research Centre and department of pulmonology, King Edward Medical University/Mayo Hospital Lahore during January 2013 to December 2016. Drug susceptibility testing from isolates of 1270 TB patients were carried out by standard drug proportion method.
Results: Drug susceptibility testing from isolates of 1270 TB patients including 759 (59.7%) males and 511 (40.3%) females was carried out by standard drug proportion method. Mean age of patients participated in this study was 40.9±17.7. Multi drug resistance was found to be 18.6% among all patients while 36.4% among patients having previous history of treatment. A total of 38.6% TB isolates were resistant to minimum of one first line anti TB drug, of which 2.4% isolates were resistant to all the four first line ATT drugs. Highest MDR TB of 25.7% was reported in 2016.
Conclusion: Monitoring of drug susceptibility testing among new and previously treated TB cases is necessary in developing countries for better understanding of disease pattern to provide prompt and appropriate treatment.
Key words: Multidrug resistance, mycobacterium, rifampicin, MDR TB.

Introduction

Global mortality and incidence rate of tuberculosis (TB) remain to drop in recent years, even then there were around 1.4 million deaths due to TB and additional 0.4 million casualties due to co-morbidity of TB among acquired immune deficiency syndrome (AIDS) patients.[1] World Health organization (WHO) has reported an estimate of 44,000 TB related deaths with a rate of around 23/100,000 population which is higher to global rate of 17/100,000 deaths.[2] The most serious problem in spread of TB is its air born transmission that makes it a considerable public health concern. The main target of SDGs is to end TB epidemic around the globe along with 90% and 80% reduction in deaths and incidence respectively in comparison with 2015.[1]

Multi drug resistant (MDR) TB had an unprecedented effect in management of TB. Patients infected from strains of Mycobacterium tuberculosis complex which are resistant to two basic anti TB drugs i.e. isoniazid (INH) and rifampicin (RIF) simultaneously with or without resistance to other first line chemotherapeutic agents are categorized to be MDR TB patients. There are multiple reasons to develop this resistance and these strains are endemic throughout the globe causing morbidity and mortality.[3] A total of 480,000 new MDR TB cases and 100,000 additional patients with RIF resistance

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Authors Contribution

RI conceptualized the project. MKM did the data collection. MKM & SR has performed literature search. RI & MKM also did the statistical analysis. Drafting, revision and writing of manuscript was done by RI, MKM & SS.

were also considered eligible for MDR TB treatment during 2015.[1,4] Pakistan ranked 5th and carries 4% of the total global burden of MDR TB patients.[5] Rate of global decline in TB incidence remained as low as 1.5% during years 2014 and 2015 which needs to be enhanced as 4-5% decline annually till 2020 to maintain primary milestone of End TB Strategy.[1]

Emergence of drug resistance (DR) has become more challenging over the last decade and created ambiguity among healthcare professionals treating this disease and chaos among patients suffering from DR TB. As drug resistant TB already limits the choice of drugs from first line anti-tubercular treatment (ATT), even worse when broad spectrum resistance including few second line drugs may occur in few strains of Mycobacterium tuberculosis complex. Any MDR TB strain resistant to fluoroquinolones and one of three injectable drugs (kanamycin, amikacin and capreomycin) may be termed as extensively drug resistant (XDR) tuberculosis.[6]

Mechanism of occurrence and spread of DR TB can be better understood by two interweaved progressions. Any TB patient seeking treatment from first line drugs of ATT and completed treatment defaulted or relapse etc. may develop drug resistance. This type of resistance is called secondary or acquired resistance while those TB patients who directly inhale bacilli from drug resistant TB patient and develop disease is categorized as primary drug resistant TB. Factors related to mechanism of occurrence of drug resistance have been extensively studied however; in the beginning patient suffering from susceptible TB is treated with standard first line regimen. Ideally quality assured ATT is provided under appropriate circumstances and assumed that patient must be cured.[7] Although failure to ATT may acquire drug resistance, many programs prescribe various cycles of first line therapy and responsible to increase the resistance against number of drugs during each, iteration of therapy and phenomenon is termed as “amplification of resistance”.[8]

Thus increase in drug resistance TB is alarming and reflecting various personal negligence level deficiencies. Despite other factors and constraints, it becomes difficult for local physicians and consultants to diagnose drug resistance as high standard laboratory infrastructure with trained and skilled manpower is lacking.[9] First TB specific antibiotic para amino salycilic acid (PAS) was discovered in 1940,s.[10] Streptomycin which was injectable drug first marketed in November 1944.[11] Isoniazid was marketed in mid of 1950,s and Rifampicin in 1970,s. with the availability of these two drugs revolution came in treatment of TB and its cure rate improved remarkably.[12] In 1980,s WHO lost its focus from tuberculosis, its after occurrence of HIV/AIDS, TB re-emerged globally and global emergency for TB was declared by WHO in 1993.[13] GeneXpert technology has been introduced for rapid diagnosis of Mycobacterium tuberculosis complex and rifampicin susceptibility testing with time duration of two hours. Programmatic management of drug resistant TB (PMDT) sites are also established in tertiary care hospitals of the country wide to enroll 100% cases of rifampicin resistance. Though, the cases with resistant to other first line drugs except rifampicin may suffer from treatment failure. Continuous monitoring of drug resistance pattern is of great importance under the given situation. Objective of current study was to see the shifting trends of drug susceptibility patterns among TB patients and to compare the frequencies of drug resistance among primary and acquired TB patients.

Materials and Methods

This descriptive study was undertaken in Pakistan Health Research Council TB Research Centre and department of pulmonology, King Edward Medical University/ Mayo Hospital Lahore during January 2013 to December 2016. Samples were collected from the patients of age 15 and above, referred for TB culture from inpatient and outpatient of all specialties of Mayo Hospital and other associated hospitals. Work was approved by the institutional review board and individual consent was obtained for participation in the study. A predesigned questionnaire was used to collect the demographic information and previous history of anti-tuberculor treatment from patients. Patients already seeking treatment from more than 4 weeks and sample contamination during culture isolation were excluded from the study.

This study comprised of 1270 cases. After isolation of Mycobacterium tuberculosis complex on Lowenstein Jensen (LJ) medium, drug susceptibility for RIF, INH, ethambutol and streptomycin was performed using standard drug proportion method. Culture grown on LJ medium containing optimum pH of 6.8 were re-suspended aseptically in 3 ml of sterile distilled water under aseptic conditions and turbidity was adjusted to 1 McFarland standard. Tenfold dilutions were prepared and two dilutions 102 and 104 were inoculated on plain media as controls while only 102 was inoculated on media containing the drugs rifampicin, isoniazid, streptomycin and ethambutol with final concentrations of 40µg/ml, 0.2 µg/ml, 4 µg/ml and 2 µg/ml respectively.

All the samples were incubated at 37°Ċ±2°Ċ for 4 weeks and number of colonies was counted from controls and drug containing media for each individual specimen. Ratio of both control and drug containing media was calculated and expressed as percentages. This semi-quantitative method provided the proportion of Mycobacterium population which is resistant to respective drug. Any isolate with ≥1% growth on rifampicin, isoniazid and ethambuol while ≥10% growth in streptomycin were labeled as resistant to corresponding drugs.

Internal Quality assurance is maintained by using American type culture control (ATCC) H37RV, known sensitive to all first line TB drugs with each batch of eleven samples. National TB Control Program share Belgium strains for drug testing to maintain external quality assurance.

Quantitative variables were presented in mean ± standard deviation while qualitative variables were presented in frequency and percentages. Chi-square test was applied to determine the level of significance between primary and acquired drug resistance among both groups and a *p* value of ≤0.05 was considered significant.

Results

Drug susceptibility testing from isolates of 1270 TB patients including 759 (59.7%) males and 511 (40.3%) females was carried out by standard drug proportion method. Mean age of patients participated in this study was 40.9±17.7. Female to male ratio of patients remained 1:1.48 and mean age of female patients (38.7±18.0) also remained low as compared to male patients (42.4±17.4).

Four first line anti TB drugs including rifampicin, isoniazid, streptomycin and ethambutol were tested. Multi drug resistance was found to be 18.6% among all patients but very high of 36.4% among patients having previous history of treatment. Highest resistance of 24.4% was observed for streptomycin and lowest of 12.7% in ethambutol.

Drug resistant pattern were compared using chi-square test among patients having previous history of ATT and no history of ATT. High drug resistance was observed in patients having previous history of ATT and significant difference was observed among all drugs with p-value of ≤0.05 as shown in Table-1.

Resistance to one drug only was observed and highest resistance of 4.6% was observed in streptomycin and lowest of 1.9% in ethambutol as shown in Table-2.

Drug susceptibility pattern among all isolates are presented in Table-3. Almost 38.6% TB isolates were resistant to minimum of one first line anti TB drug of which 2.4% were isolates were resistant to all the 4 first line drugs tested in this study.

Table 1: Pattern of first line drugs resistance among primary and acquired tuberculosis patients.

Drug Resistant to	*All Patients N = 1270 n (%)*	*No Treatment History N = 803 n (%)*	*Treatment History N=467 n (%)*	*p-value*
Isoniazid + Rifampicin (MDR)	236 (18.6)	66 (8.2)	170 (36.4)	0.0001
Rifampicin	290 (22.8)	80 (9.9)	210 (44.9)	0.0001
Isoniazid	295 (23.3)	82 (10.2)	213 (45.6)	0.0001
Streptomycin	309 (24.4)	87 (10.8)	222 (47.5)	0.0001
Ethambutol	161 (12.7)	45 (5.6)	116 (24.8)	0.0001

Table 2: Mono resistance pattern. (N = 1270)

Drug	*F*	*%*
INH	48	3.7
Rif	51	4.0
ETB	25	1.9
STR	59	4.6

The year wise distribution of drug resistance and number of cases tested per year are shown in Table-4. Highest number of isolates was inoculated for drug susceptibility testing in year 2014 while maximum frequency of 25.7% of MDR TB cases was observed in year 2016 while lowest of 16.0% was observed in year 2015.

Table 3: Drug susceptibility pattern with reference to the number of resistant drugs in all samples. (N = 1270)

Susceptible to Drugs	F	%
Susceptible to all test drugs	780	61.4
Resistance to single drugs	189	14.9
Resistance to two drugs	148	11.6
Resistance to three drugs	122	9.6
Resistance to four drugs	31	2.4

Discussion

Present study revealed multi drug resistance among 18.6% isolates remained 2.0% higher as compared to previous study from same settings.[9] A thirteen year study from this institute also reported 15.7% multi drug resistance among

Table 4: Yearly distribution of drug resistance.

Drugs	History of ATT	Year				Total N=1270 n (%)
		2013 N=274 n (%)	2014 N=385 n (%)	2015 N=374 n (%)	2016 N=237 n (%)	
Isoniazid + Rifampicin (MDR)	Present	39 (14.3)	44 (11.4)	44 (11.8)	43 (18.2)	170 (13.4)
	Absent	13 (4.8)	19 (4.9)	16 (4.3)	18 (7.6)	66 (5.2)
	Total	52 (19.0)	63 (16.4)	60 (16.0)	61 (25.7)	236 (18.6)
Rifampicin	Present	42 (15.3)	51 (13.3)	58 (15.5)	59 (24.9)	210 (16.5)
	Absent	16 (5.8)	23 (6.0)	22 (5.9)	19 (8.0)	80 (6.2)
	Total	58 (21.2)	74 (19.3)	80 (21.4)	78 (32.9)	290 (22.8)
Isoniazid	Present	51 (18.6)	57 (14.8)	57 (15.3)	48 (20.3)	213 (16.7)
	Absent	21 (7.6)	24 (6.3)	20 (5.4)	17 (7.2)	82 (6.5)
	Total	72 (26.2)	81 (21.1)	77 (20.6)	65 (27.4)	295 (23.3)
Streptomycin	Present	66 (24.0)	52 (13.5)	57 (15.3)	47 (19.8)	222 (17.4)
	Absent	25 (9.2)	18 (4.7)	30 (8.1)	14 (5.9)	87 (6.9)
	Total	91 (33.2)	70 (18.2)	87 (23.3)	61 (25.7)	309 (24.4)
Ethambutol	Present	27 (9.9)	34 (8.8)	32 (8.6)	23 (9.7)	116 (9.2)
	Absent	9 (3.3)	12 (3.1)	13 (3.5)	11 (4.7)	45 (3.5)
	Total	42 (15.3)	50 (13.0)	45 (12.1)	40 (16.9)	161 (12.7)

isolates few years back,[14] hence gradual increase in MDR TB has been observed. Occurrence of MDR TB among re treatment case in this study was 36.4% remained very low as compared to another study from Punjab, Pakistan that reported prevalence of MDR TB as 69% among retreatment cases.[15] Results of current study are not comparable with an older study that presented prevalence of MDR TB as 30.7% with an increase of 2% from their own earlier study.[16] Another study from Aga Khan University Karachi has also reported high prevalence of 38.7% and alarming gradual increase in prevalence of XDR TB as 1.5% in year 2006 to 4.5% in 2009.[17] Similarly a study from India has reported 20.4% MDR TB among category II TB patients.[18].

Primary MDR TB of 8.2% in present study remained low as compared to previous studies from same settings in which it was 12.8% and 12.3% respectively.[9,14] Reasons behind this high frequency of primary MDR TB were narrated as low literacy rate at patients end and unavailability of central or computerized record keeping at institutional level.[9] Although we have tried our best to cope with the issues of record keeping at institutional level still there may be the cases in category of no treatment may truly be the re-treatment cases and fall in category of primary MDR TB instead of acquired MDR TB, as such observations have been made by physicians in outpatients and PMDT site of the institution. Global rate of primary MDR TB was reported to be 3.7% by WHO[19] in 2012 is not in agreement with current study however data varied in range of 5.4% to 28.3% of primary MDR TB[20] support results of current study.

Mono drug resistant is also very interesting phenomenon when we talk about TB. Isolates from 14.9% patients were resistant to only one of the four drugs tested in present study. Further, in isolation, streptomycin showed highest resistance of 4.6% followed by rifampicin 4.0%, isoniazid 3.7% and ethambutol 1.9%. There are only five drugs used as first line of ATT. In new TB patients; isoniazid, rifampicin, pyrazinamide and ethambutol are given to the patient for intensive phase of two months and isoniazid and rifampicin are given for four months of continuation phase. In TB patients with history of ATT, treatment defaulters and relapse, streptomycin is added along with all four drugs for two months, streptomycin is discontinued from 3rd month of treatment and pyrazinamide is also excluded from 4th month in a total treatment period of 8 months.[21] There is already very limited choice to make combination as few of the drugs are more toxic and long term use may be lethal for patients.[21] Factors behind mono resistance belong to understanding of disease at physician level while frequent change of physician and institutions at patient level.[9]

Mono resistance of 4.0% for rifampicin in present study is important as it has been acclaimed surrogate marker for MDR TB because it is assumed that 90% of these cases also resistant to isoniazide[22] however, agreement rate of isoniazid resistance in rifampicin resistance is around 80% in this study. Hence the MDR TB treatment is started

on the basis of rapid GeneXpert test for rifampicin resistance but WHO does not allow omitting the necessity of conventional tests including drug susceptibility testing. Therefore, this test is not recommended for monitoring of treatment due to its ability to detect both dead and live bacilli.[23] Mean age of 40.9±17.7 suggests that majority of patients suffer from TB lie in their best productive age as shown in other studies.[15,24] Female to male ratio of 1:1.48 also suggest high frequency of infection among males who are the main earning units of family again in agreement with other studies.[15,24]

In conclusion, monitoring of drug susceptibility testing among new and previously treated cases is necessary in developing countries for better understanding of disease pattern to provide prompt treatment. Multi drug resistance is on the rise and 2% increase in this type of TB is seen from previous study in same settings. Although National and Provincial TB reference laboratories are established country wide but facility of susceptibility testing is very much limited. It is suggested under the light of current study that existing infra-structure may be utilized to upgrade and strengthen the laboratories with provision of permission to do TB drug susceptibility testing at multiple centers throughout Pakistan for generation of national data.

Conflict of interest: None declared.

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